510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
k041040

B. Purpose for Submission:
New device

C. Analyte:
Anti-neutrophil cytoplasmic antibody (ANCA)

D. Type of Test:
Semi-quantitative and qualitative ELISA

E. Applicant:
Sweden Diagnostics (Germany) GmbH

F. Proprietary and Established Names:
Varelisa® MPO ANCA

G. Regulatory Information

1. Regulation section:
   21 CFR § 866.5660, Multiple Autoantibodies Immunological Test System
2. Classification:
   Class II
3. Product Code:
   MOB, Anti-neutrophil cytoplasmic antibody (ANCA)
4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   The Varelisa MPO ANCA EIA kit is designed for the semi-quantitative and qualitative determination of myeloperoxidase anti-neutrophil cytoplasmic antibodies (MPO ANCA) in human serum or plasma to aid in the diagnosis of certain autoimmune vasculitides such as microscopic polyangiitis, and crescentic glomerulonephritis.
2. Indication(s) for use:
   This is used as an aid in the diagnosis of certain autoimmune vasculitides such as microscopic polyangiitis, and crescentic glomerulonephritis.
3. Special condition for use statement(s):
   The device is for prescription use only.
4. Special instrument Requirements:
   A microplate reader capable of measuring OD at 450 nm is required.

I. Device Description:

The device is an enzyme-linked immunosorbent assay (ELISA) using microtiter plates as the solid phase. The plate wells are coated with MPO antigens, which allow anti-MPO antibodies to react with the immobilized antigen (sample). The conjugate is rabbit anti-human IgG horseradish peroxidase (HRP), which uses 3, 3'5, 5' tetramethylbenzidine dihydrochloride (TMB) as substrate. The kit contains a set of

six calibrators, low positive, high positive and negative controls. The kit also contains sample diluent, wash buffer concentrate and stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   INOVA Quanta Lite MPO
2. Predicate K number(s):
   k955022
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	Varelisa® MPO ANCA	Quanta Lite™ MPO
Intended Use	To aid in the diagnosis of certain autoimmune vasculitides such as microscopic polyangiitis and crescentic glomerulonephritis	To aid in the assessment of certain autoimmune vasculitides such as microscopic polyarteritis and crescentic glomerulonephritis
Antigen	Human purified MPO antigen	Same
Conjugate	HRP conjugated anti-human IgG antibody	Same
Assay principle	Indirect noncompetitive enzyme immunoassay	Same
Sample dilution	1:101	Same
Differences		
Item	**Device**	**Predicate**
Specimen requirements	Serum and plasma	Serum specimen
Calibrators	Set of six prediluted calibrators	None
Controls	Low positive, high positive and negative controls	Prediluted low positive and high positive controls
Result interpretation	Semiquantitative <6 U/mL = negative 6-9 U/mL = equivocal >9 U/mL = positive Qualitative Ratio <1.0 = negative Ratio 1.0-1.4 = Equivocal Ratio >1.4 = Positive	Semiquantitative <20 U/mL = negative 21-30 U/mL = weak positive >30 U/mL = positive

K. Standard/Guidance Document Referenced (if applicable):

None referenced

L. Test Principle:

The assay is an indirect noncompetitive enzyme immunoassay. The wells of a microtiter plate are coated with human purified MPO antigen. Antibodies specific for MPO present in the patient samples bind to the antigen. In a second step, the enzyme labeled second antibody (conjugate) binds to the antigen-antibody complex which leads to the formation of an enzyme labeled conjugate-antibody-antigen complex. The enzyme labeled antigen-antibody complex converts the added substrate to form a colored solution. The rate of color formation from the chromogen is a function of the amount of conjugate complexed with the bound antibody and thus is proportional to the initial concentration of antibodies in the patient sample.

M. Performance Characteristics (if/when applicable)

1. Analytical performance:

a. Precision/Reproducibility:

The purpose of the precision study was to investigate variation within and between runs. The samples (low, medium and high) were used in a standard 1:101 dilution except sample QC6 which was diluted 1:117. These diluted samples were analyzed in 5 runs with 16 replicates per run. Calibrators and controls were analyzed in duplicate. One operator carried out the analyses within one day. The following target values were set:

Variance within <10%
Variance between <15%

The specifications were met and results are summarized below:

Sample ID	Mean U/mL	Within-Run %CV	Between-Run %CV
QC2	9.8	9.5	6.5
QC3	25.1	6.6	10.7
QC6	77.4	4.8	3.5

Reproducibility study was expanded using two additional samples around the cut-off (1 negative and 1 equivocal sample). For equivocal or positive samples, the percents within and between variance were <10% and <15% respectively and the negative samples should not become positive. The data showed that the between and within assay variations were within stated specifications and the values for negative samples did not change into positive.

Sample	Run 1	Run 2	Run 3	Run 4	Run 5	Mean U/mL	Variance Within %CV	Variance Between %CV
Negative	5.5	6.3	6.2	5.9	5.8	5.9	15.7	3.9
Equivocal	7.2	7.8	7.8	7.5	7.3	7.5	8.3	3.2

b. Linearity/assay reportable range

Dilution Linearity

The reportable range of 1-100 U/mL was demonstrated by performing dilution study over the whole measuring range. Beginning with a standard dilution of 1:101, the samples were further diluted 2:3, 1:2, 1:4, 1:8, 1; 16, and 1:32 using sample diluent. Calibrator, controls and each dilution step were analyzed in triplicates. Specifications set for the study were the percents of observed/expected (O/E %) should be within ±20% for at least 3 successive dilution steps of each tested sample. Measured values ranged from 2.8 to 95.7 U/mL. Percent recovery for all six samples through the 1:32 dilution met the specifications.

Recovery

The purpose of this study was to demonstrate that the assay can detect known amounts of MPO specific IgG antibodies spiked into a sample. Two samples were diluted 1:101 and 1:201 respectively. Then the samples were spiked with 1/10 volume of calibrators S1-S6. The spiked samples, calibrators and controls were analyzed in duplicate. The Δ recovery (%) of (observed value/expected value) for the samples should be within ±20%. Recovery values ranged from 94.8% to 101.1 % demonstrating that the assay detects added amounts of antigen specific IgG antibodies.

c. *Traceability (controls, calibrators, or method):*
An international reference material for anti-MPO antibodies is not available. The assay is calibrated in relative arbitrary units (U/mL).

d. *Detection limit:*
The purpose of the analytical sensitivity was to verify the detection limit of the assay and to prove the ability of the assay to differentiate between the background and the first calibrator point. The sample diluent was diluted 1:101 and measured 56 times on one plate. Calibrators and controls were analyzed in four replicates. The value for the analytical sensitivity expressed in U/mL was calculated as the mean of the optical densities (OD) of the sample diluent plus 3 SD. The mean OD plus 3 SD of the sample diluent should be lower than the mean OD of calibrator S2. The detection limit of the device is 1.0 U/mL

e. *Analytical specificity:*
Interference:
Interference study was performed by spiking three samples with different amounts of potentially interfering substances including, bilirubin (F and C), hemoglobin, chyle and rheumatoid factor or their respective blank solutions and analyzed in triplicates. Calibrators and controls were also analyzed in triplicate. The deviation of the value of the sample spiked with the interfering substance should be less than ± 20% of the value of the sample spiked with a buffer blank. The spiking of high concentrations of Bilirubin C, Bilirubin F,

Chyle, Hemoglobin and Rheumatoid Factor showed no significant effect on the test results.

Cross Reactivity:

Quanta Check ANCA Panel from INOVA was used in this study.

Serum	Varelisa result (U/mL)	Target (according to INOVA)	Reactivity against MPO (Results according to INOVA)
Inova A	5.7	cANCA	negative
Inova B	0.2	PR-3	negative
Inova C	**64.3**	**pANCA**	**positive**
Inova D	**>100**	**MPO**	**positive**
Inova E	6.7	pANCA & aANCA*	negative
Inova F	0.5	aANCA*	negative
Inova G	0.4	ANA	negative
Inova H	0.4	negative	negative

* Atypic ANCA, not positive for MPO or PR-3

The test results showed no crossreactivity to PR-3 or ANA antibodies.

f. Assay cut-off:

A study was performed to confirm the defined cut-off by measuring 432 apparently healthy blood donor samples, equally distributed by gender and age. Specifications for the study were: 95% of the normal population should be negative and the 95th percentile should lie below the lower limit of the equivocal range. The cut-offs were set as

<6 U/mL negative
6-9 U/mL equivocal
>6 U/mL positive

The 95th percentile was 3.4 U/mL and thus below the negative cut-off so, the specifications were met. The results were independent of gender and age.

2. Comparison studies:

a. Method comparison with predicate device:

The comparison was made by testing 270 clinically defined sera. (15 Churg-Strauss Syndrome, 50 microscopic polyangiitis, 20 non-ANCA associated vasculitides, 16 necrotizing crescentic glomerulonephritis, 40 rheumatoid arthritis, 102 Wegener's granulomatosis, 10 Morbus Crohn, 10ulcerative colitis, 7 other diseases). Analyses and calculations were performed according to assay procedures. Correlations and Six Field Analysis were performed.

	N = 270	**INOVA QUANTA Lite**	
		Positive	Negative
Varelisa MPO ANCA	positive	34	13**
	Equivocal	1***	19****
	negative	1*	202

*1 patient with microscopic polyangiitis (MPA)
**12 ANCA associated vasculitides (AAV) and 1 disease control
***1 disease control
****19 AAV

The equivocal results of the Varelisa™ MPO ANCA were regarded as negative in calculation of percent agreements.
Positive agreement = 94.4% (95% CI 81.3% to 99.3%)
Negative Agreement = 94.4% (95% CI: 90.7% to 97.0%)
Total Agreement = 94.4% (95% CI 91.7% to 97.1%)

b. Matrix comparison:
The predicate device uses serum only. The new device recommends use of both serum and plasma. A study was performed to demonstrate that the new assay gives the same results for serum, heparin plasma, citrate plasma, and EDTA plasma collected from the same specimen. Ten MPO-antibody negative samples and 10 MPO-antibody positive samples, each available as serum, heparin plasma, citrate plasma and EDTA plasma variant were assayed. The 10 MPO-antibody negative samples were run in duplicate together with calibrators and controls. Then they were spiked with the 10 different MPO-antibody positive sera. All spiked samples were run in duplicate together with calibrators and controls. Specifications for this study were the percent deviation between serum and plasma results for positive samples should not be higher than ±20% and negative samples should be negative as serum or plasma. The data showed no difference greater than ±20% (deviations ranged from –16.4% to 13.6% for citrate, heparin or EDTA plasma and no negative sample changed from negative to positive). Thus the specifications were met.

3. Clinical studies:
   a. Clinical sensitivity:
   Not provided
   b. Clinical specificity:
   Not provided
   c. Other clinical supportive data (when a and b are not applicable):
   Not applicable.
4. Clinical cut-off:
   See assay cut-off
5. Expected values/Reference range:
   The expected value in the normal population is negative.

N. Conclusion:

The submitted material in this premarket notification is complete and supports a substantial equivalence decision.